

IN THE NAME OF GOD



Qazvin University
Of Medical Sciences

Biosensor-based clinical diagnosis

molecule

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□ overview

➤ Introduction

➤ Discussion

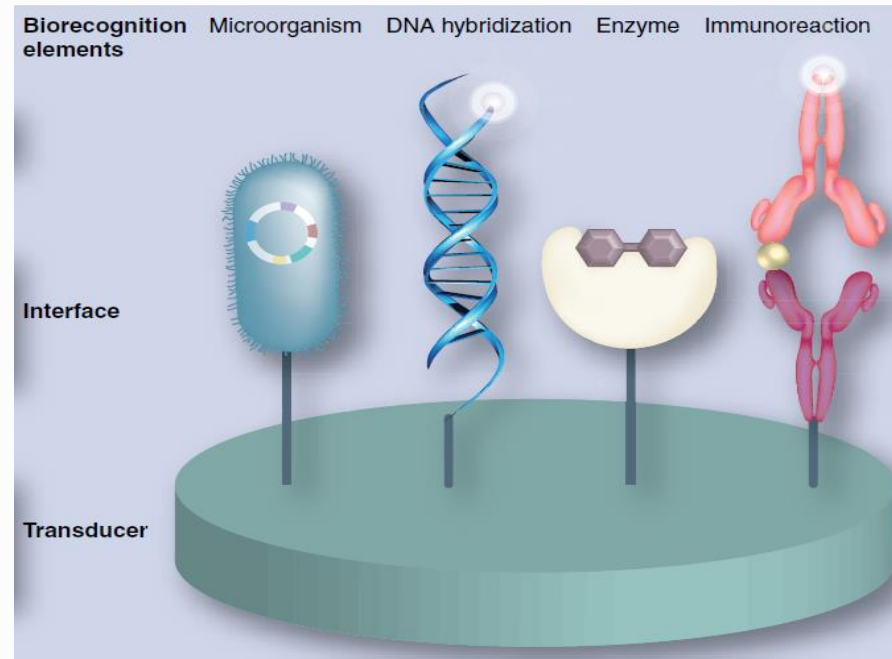
➤ Conclusion

➤ Reference



□ Introduction [1]

- **Biosensor**: As an analytical device that can diagnose the interest analyte in a sample and measure it quantitatively
- Consisting of a **biological recognition** element and a **physical transducer**



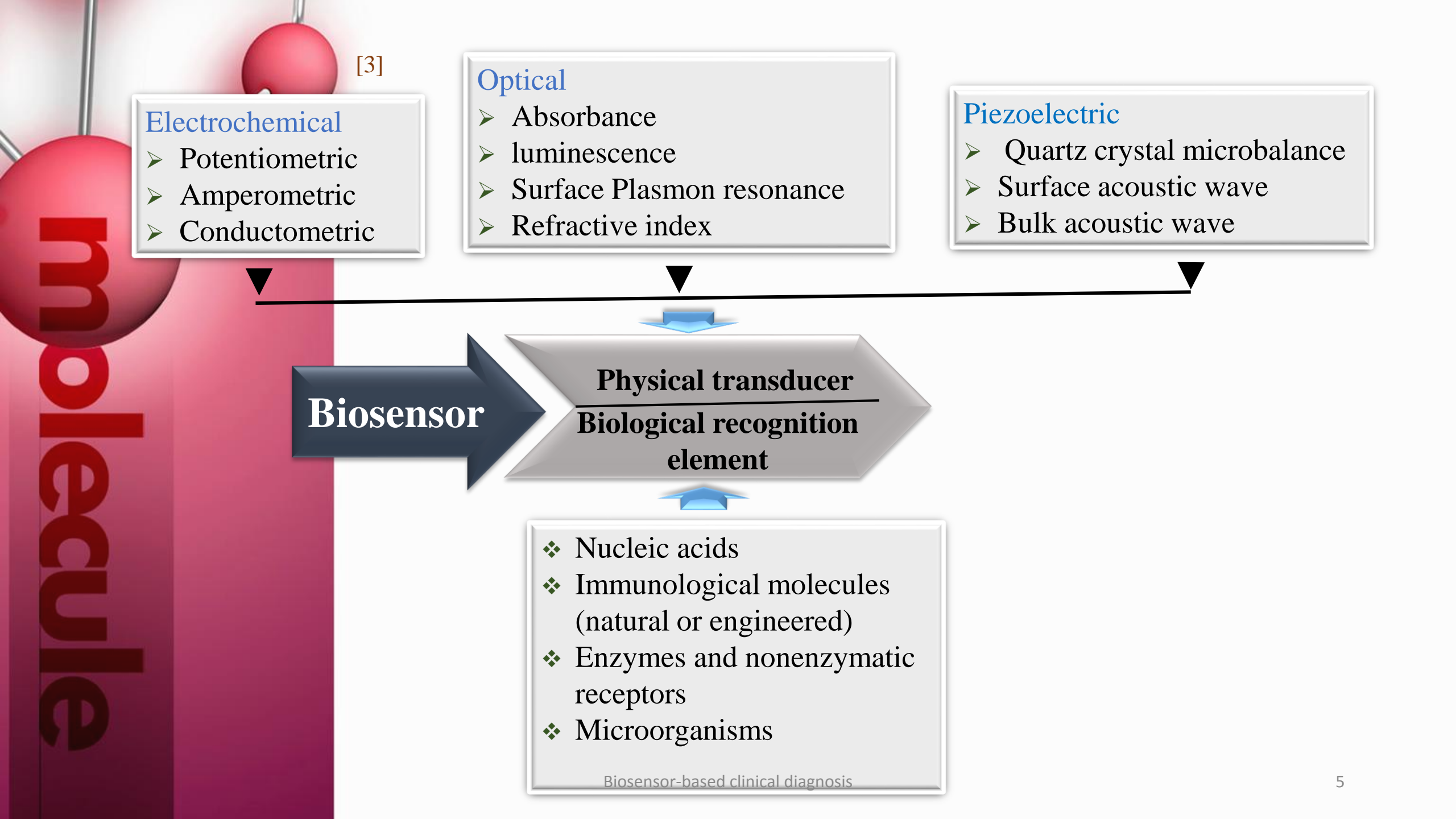


□ Introduction ^[1,2]

❖ **Biorecognition element**: responsible to specific recognition of an analyte of interest

❖ **physical transducer**: converts the biorecognition event into a measurable **optical** or **electric** signal

- With wide range of applications, such as:
 - ✓ Clinical diagnosis
 - ✓ Environmental monitoring
 - ✓ Food safety, drug discovery
- The **first biosensor** (glucose biosensor) invented by Clark and Lyons (1962)





❑ Biorecognition element immobilization techniques in biosensor [4]

- Microencapsulation
- Entrapment in gel
- Crosslinking
- Covalent immobilization
- Physical adsorption



❑ Biorecognition element immobilization techniques in biosensor [4]

1. Microencapsulation:

Use an **ineffective membrane** (cellulose acetate, polycarbonate, collagen)

2. Entrapment in gel:

The biological material mixture with a solution of monomers then monomers polymerized to becomes gel(Polyacrylamide)



□ Biorecognition element immobilization techniques in biosensor

3. Crosslinking:

Biological material bind to a solid carrier or materials such as gel connected (multifunctional material such as glutaraldehyde)

4. Physical adsorption:

Exploiting **non-covalent** interactions (hydrophobic forces, ionic binding, hydrogen bonding, and van der Waals interactions)



❑ Biomolecule immobilization techniques in biosensor

5. Covalent immobilization:

- Involves direct **covalent binding** (amine coupling and thiol coupling)
- Use working surfaces:
 - ✓ Metals (gold, silver...)
 - ✓ Carbonaceous surfaces (graphene, glassy carbon)



❑ Biosensors classification [2,3]

❖ Depending on transducers nature

- ✓ Electrochemical biosensors
- ✓ piezoelectric biosensors
- ✓ Optical biosensors

❖ Depending on recognition element nature

- Immunosensor
- Nucleic acid-based biosensor
- enzyme biosensors
- Aptasensor



❑ Biosensors depending on recognition element nature [5]

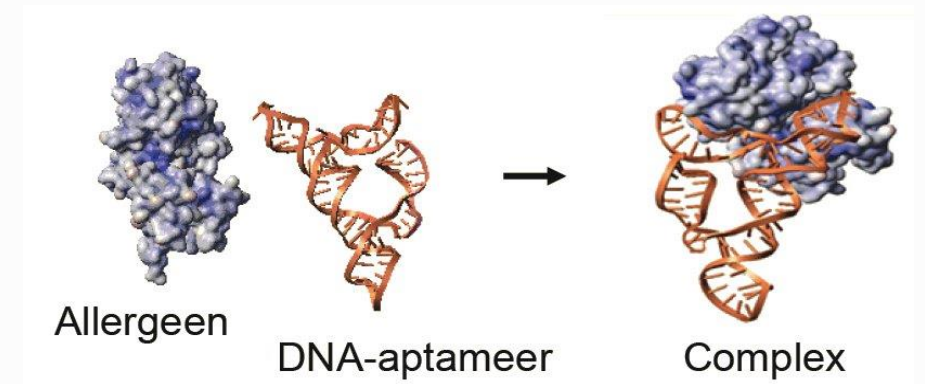
- **Immunosensor:** specific interaction of **antibody** with antigenic regions of an analyte (antibody as a biorecognition)
- **Nucleic acid-based biosensor :** the hybridization of known molecular **Nucleic acid probes** or sequences with complementary strands in a test sample
- **Enzyme biosensors:** relies upon a natural or fortuitous specificity of given enzymatic protein to react biochemically with a target substrate

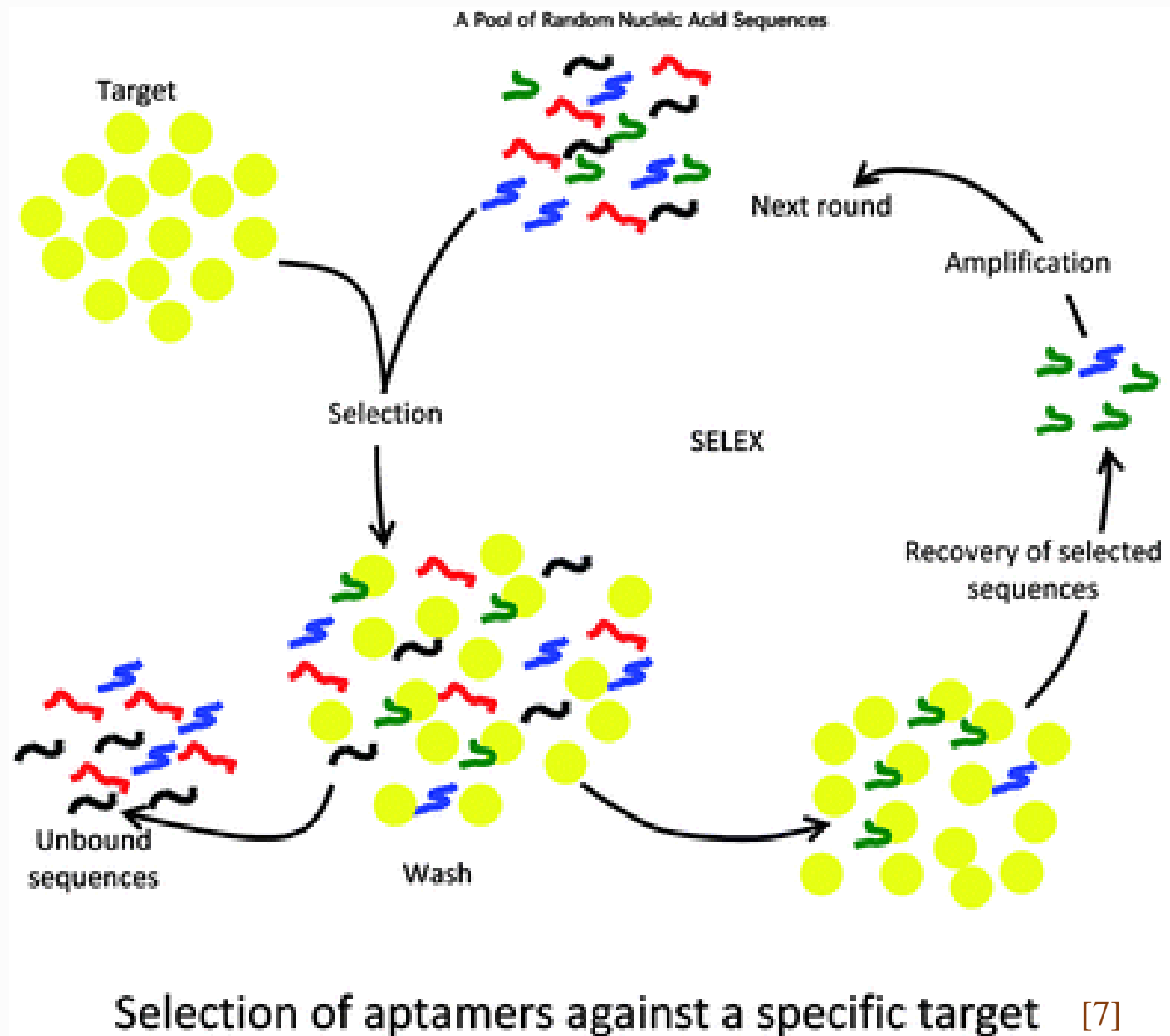
- Aptasensors: biosensors that the **biorecognition** section consist of **aptamer** [6]

□ Aptamer

- ❖ Single-stranded synthetic (RNA or DNA) oligonucleotide
- ❖ Separation from **aptamer libraries** by **SELEX** Process
- ❖ Formation different specific **three-dimensional** structures
- ❖ **Shape complementary** binding to target

SELEX :systematic Evolution of Ligands by Exponential Enrichment



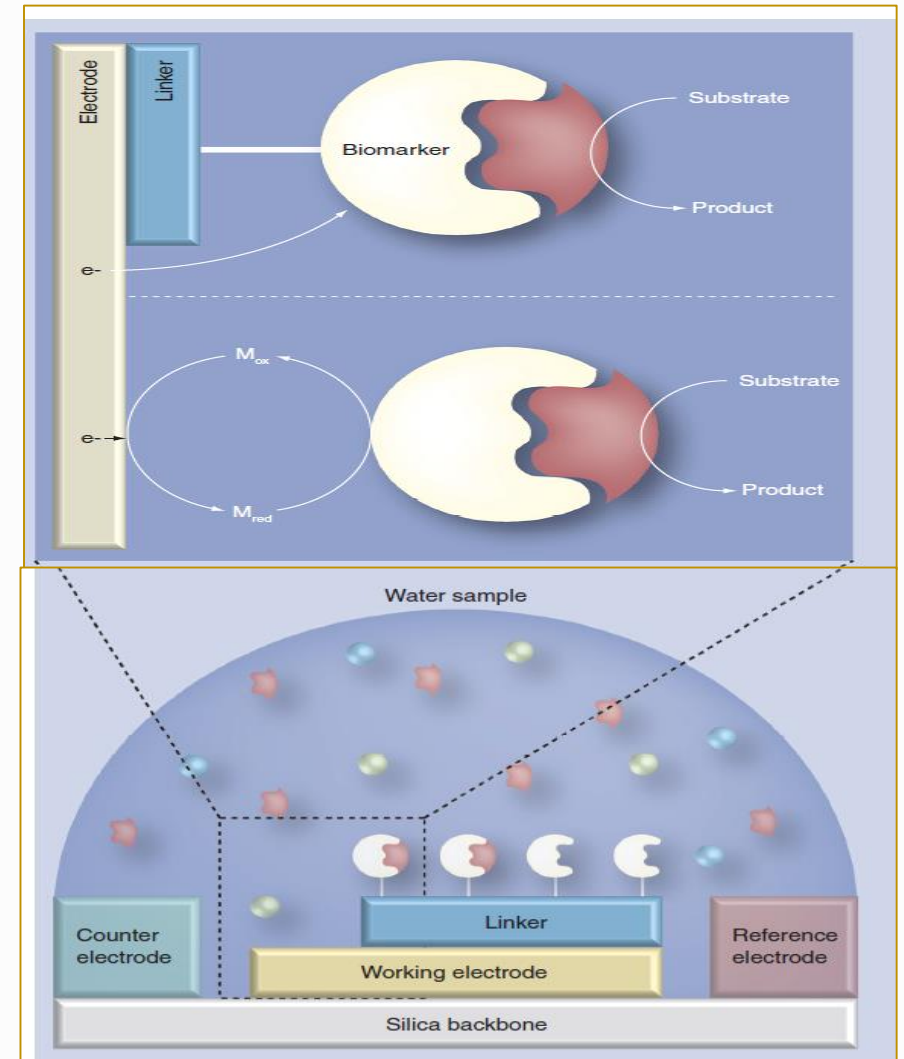




Biosensors depending on transducers nature

❑ Electrochemical biosensors [8]

- Use **electrode** (gold , platinum, nickel) as transducer
- Enzyme electrochemical biosensors (**oxidoreductase** enzymes)
- Affinity electrochemical biosensors (NA,Ab...) labeled by an enzyme (phosphatase, peroxidase)





❑ Electrochemical biosensors

- The biorecognition- analyt interaction cause change in **potential**(potentiometric) or **current**(amperometric) on **electrode surface** as output signal
- As a function of, target analyte **type** or **concentration**



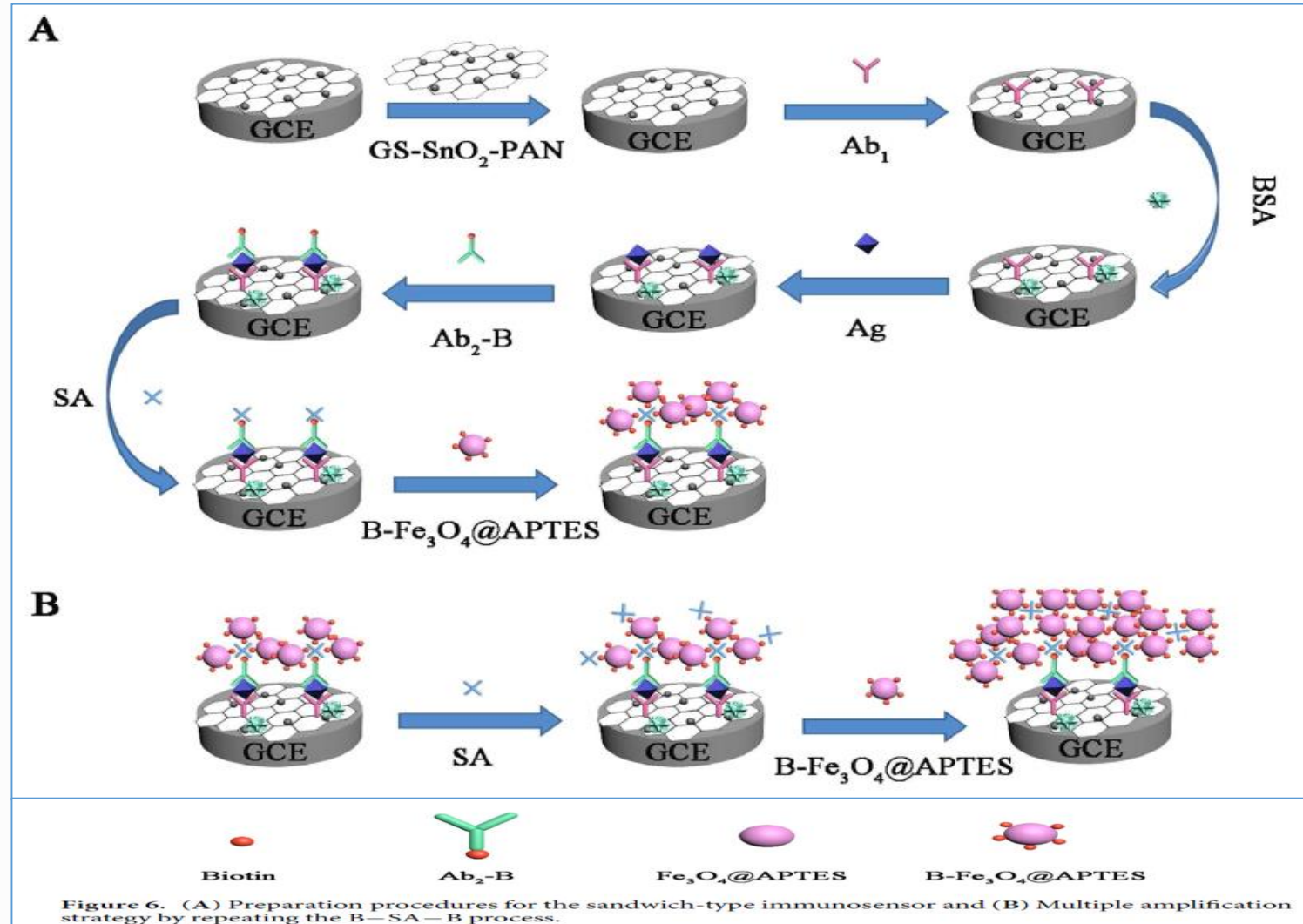
□ Electrochemical biosensors [9]

A sandwich-type electrochemical immunosensor based on the biotin- streptavidin-biotin structure for detection of human immunoglobulin G

Yueyun Li^{1,2}, Yihe Zhang¹, Liping Jiang², Paul K. Chu³, Yunhui Dong² & Qin Wei⁴

A sandwich-type immunosensor is designed and fabricated to detect the human immunoglobulin G (HlgG) using polyaniline and tin dioxide functionalized graphene (GS-SnO₂-PAN) as the platform and biotin-functionalized amination magnetic nanoparticles composite (B-Fe₃O₄@APTES) as the label. GS-SnO₂-PAN is used as the sensing agent to capture the primary anti-HlgG (Ab₁) and SnO₂ reduces the stack of GS. The B-Fe₃O₄@APTES with a large surface area and excellent biocompatibility captures second antibody (Ab₂) efficiently based on the highly selective recognition of streptavidin to biotinylated antibody. The B-Fe₃O₄@APTES has better electro-catalytic activity in the reduction of hydrogen peroxide (H₂O₂) and the "biotin-streptavidin-biotin" (B-SA-B) strategy leads to signal amplification. Under optimal conditions, the immunosensor has a wide sensitivity range from 1 pg/L to 10 ng/L and low detection limit of 0.33 pg/L (S/N = 3) for HlgG. The immunosensor has high sensitivity, fast assay rate, as well as good reproducibility, specificity, and stability especially in the quantitative detection of biomolecules in serum samples.

□ Electrochemical biosensors





❑ Piezoelectric biosensors

[10,11]

- ❖ An acoustic wave or piezoelectric biosensor utilizes **acoustic** or **mechanical waves** as a detection mechanism
- ❖ Mass sensitive biosensors
- ❖ Made with thin piece of **piezoelectric crystals** such as quartz, lithium niobate, or lithium tantalite (generate acoustic wave)
- ❖ Consist of a parallel electrode (transducer) placed on both sides of the crystal



❑ Piezoelectric biosensors

- ❖ **Piezoelectric effect:** production of electrical charges by the imposition of mechanical stress
- ❖ **Converse effect:** Applying an appropriate electrical field to a piezoelectric material creates a mechanical stress



□ Piezoelectric biosensors

Applying electrical field



Excited acoustic waves



Mechanical oscillation of acoustic wave



Change in oscillation frequency by analyte binding



Electrically measurement changes in mass ,elasticity, conductivity
in associate with Mass(analyt)attaches on crystal surface



□ Piezoelectric biosensors [12]

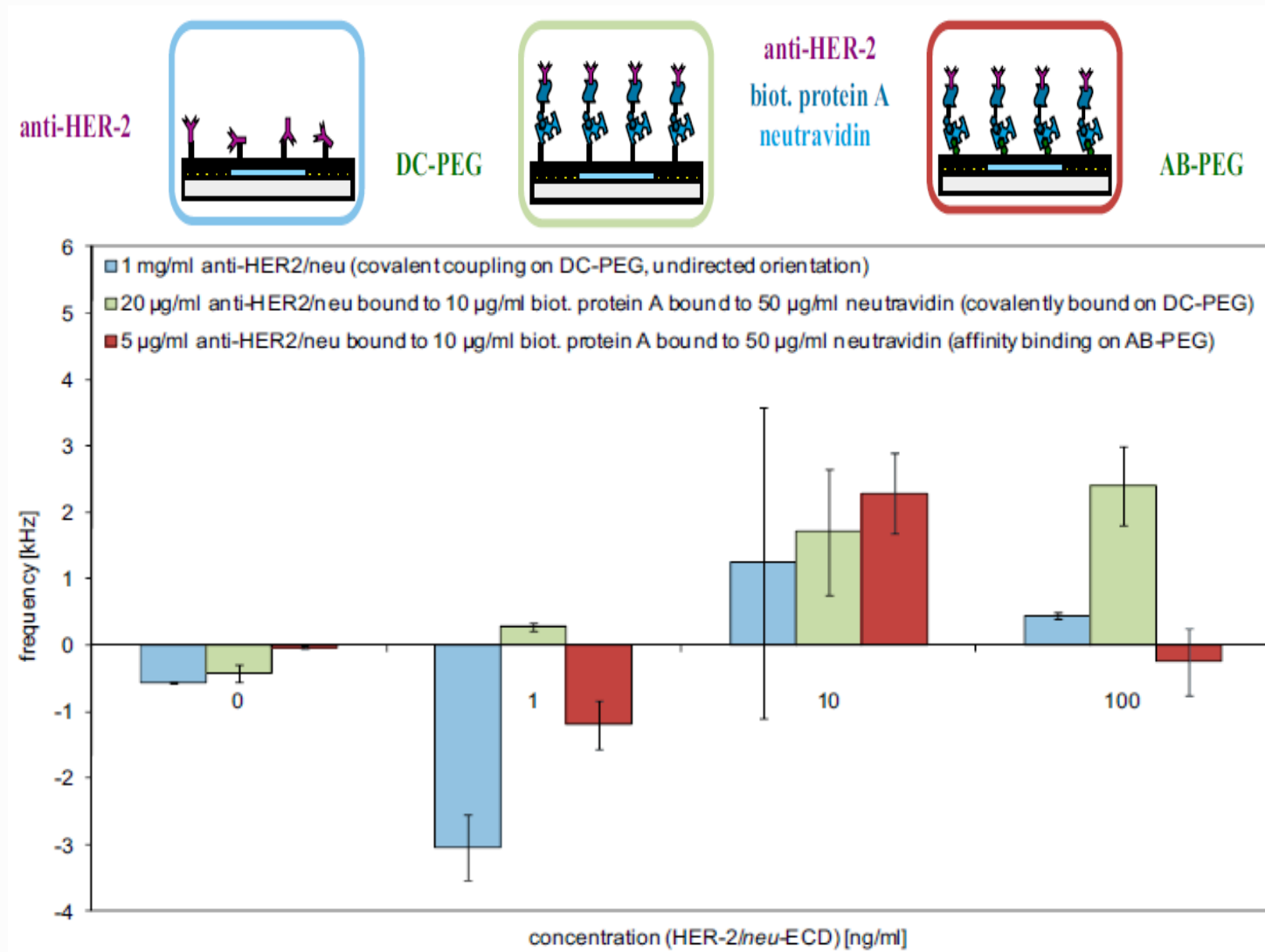
Abstract

Surface acoustic wave (SAW) biosensors based on horizontally polarized surface shear waves enable label-free, sensitive and cost-effective detection of biomolecules in real time. Binding reactions on the sensor surface are detected by determining changes in surface wave velocity caused mainly by mass loading in the sensing layer. Typically, SAW devices are coated with biochemically sensitive layers including analyte-specific capture molecules (e.g., antibodies) or ligands. The covalent binding of antibodies to intermediate hydrogel layers (e.g., dextran or polyethylene glycol) tends to result in undirected orientation of capture molecules and leading to a lower signal response in a subsequent analyte binding experiment. Therefore, a coupling procedure was developed using two linkers, neutravidin and biotinylated protein A, allowing directed orientation of capture antibodies. This assembly enables label-free and direct detection of the breast cancer marker HER-2/*neu* at a concentration of 10 ng/ml (threshold: 13-20 ng/ml).

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Keywords: biosensors; label-free; immobilization; breast cancer; HER-2/*neu*

□ Piezoelectric biosensors [12]



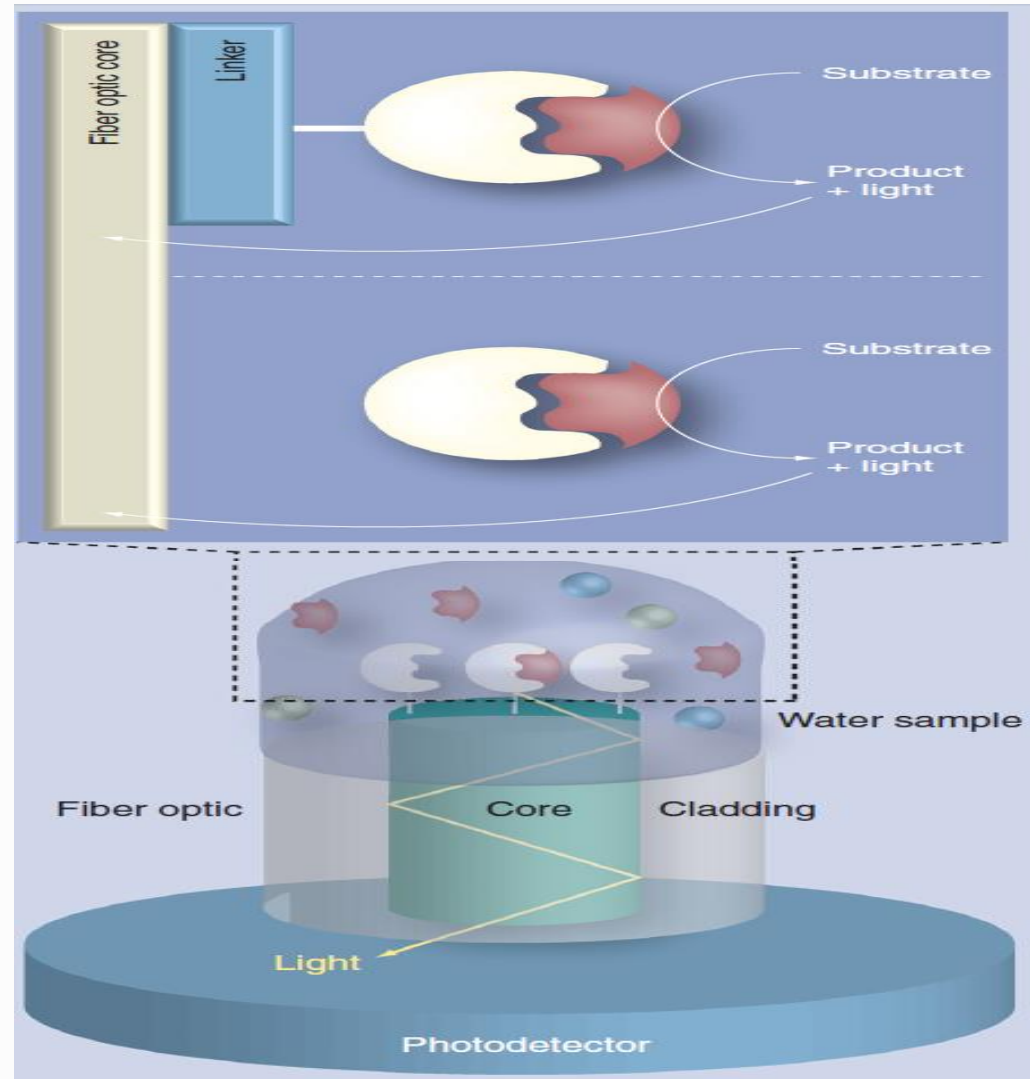


❑ Optical biosensors [2]

- Based on the measure changes of the incident light, arise the bioreceptor-target interaction
- Measured changes in :
 - ✓ Fluorescence
 - ✓ Surface plasmon resonance or refractive index
 - ✓ Absorbance
- Use fiber optic as transduction element made of glass or plastic



Optical biosensor





❑ Fluorescence- based optical biosensors [14,15]

- ❖ Fluorescence: the process of **light emission** from excited species(Fluorophore)
- ❖ The **fluorescent markers** (coupled with bioreceptor) are excited by the incidental light from the evanescent wave
- ❖ Fluorescent **intensity** , as a function of biosensor-target interaction as well as **target molecule concentration**



□ Fluorophores [14.15]

1. Nanomaterials

- ❖ Semiconductor **quantum dots**
- ❖ Nanoscale metal clusters
- ❖ Organo-mineral nanocomposites

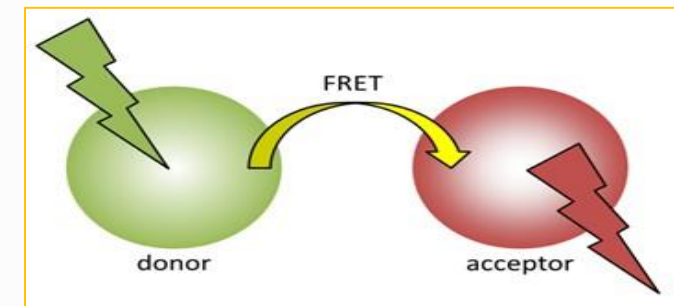
2. Molecular compound

- Organic compounds
 - ✓ Aromatic hydrocarbons (naphthalene, phenanthrene)
 - ✓ Amino acids (tryptophan, phenylalanine, tyrosine)
 - ✓ Fluorescein, rhodamine
- Inorganic compounds:
 - ✓ Uranyl ion (UO_2^{+})
 - ✓ Lanthanide ions (Tb^{3+} and Eu^{3+})
- Organo-metallic Compounds
 - ✓ 8-hydroxy quinoline

❑ FRET-based biosensors [16]

- **FRET** : a nonradioactive **energy transfer process** between two Fluorophores(**donor and acceptor**) located in close proximity to each other
- FRET-based biosensors **genetically encoded**
- Performance in **live cell**
- Used to visualize activity of cellular **signaling molecules** such as:
 - Ca^{2+} , phospholipids
 - Small GTPase, protein kinases

FRET: Fluorescence resonance energy transfer





❑ Main types of FRET fluorophores [17]

➤ Quantum dots

➤ Fluorescent proteins (FPs)

❑ Cyan FP-yellow(CFP-YFP) Pairs

❑ Green-red(GFP-RFP) FRET Pairs

❑ Far-red FPs (FFPs) and infrared fluorescent proteins (IFPs)

➤ FRET **biosensors efficiency** determined by:

➤ Fluorophores **distance** ,orientation

➤ Proper **spectral overlap** of the donor emission and acceptor excitation,




❑ FRET efficiency measurement methods: [17]

1. Ratiometric analysis

Measure the acceptor/donor intensity ratio

2. lifetime analysis

Measure donor lifetime change

Two type of FRET biosensor: 

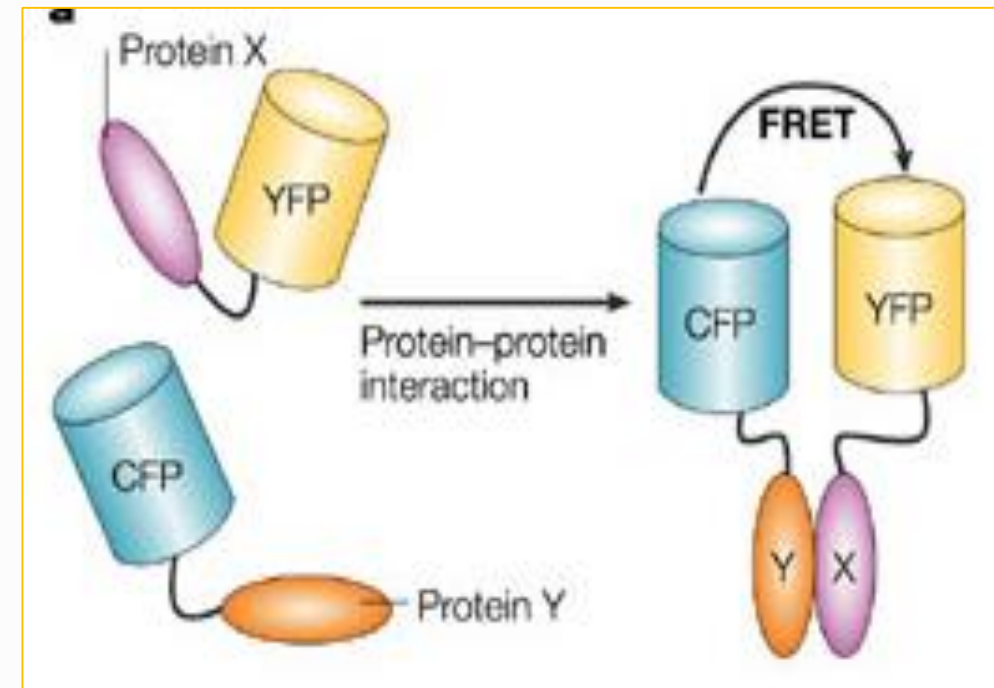
Intermolecular biosensor

Intramolecular biosensor



❑ Intermolecular biosensor [18]

- ❑ Donor and acceptor fluorophores fused to **different molecules**
- ❑ When two protein interact, bring the fluorophores into close proximity ,so increase the FRET efficiency





□ Intramolecular biosensor

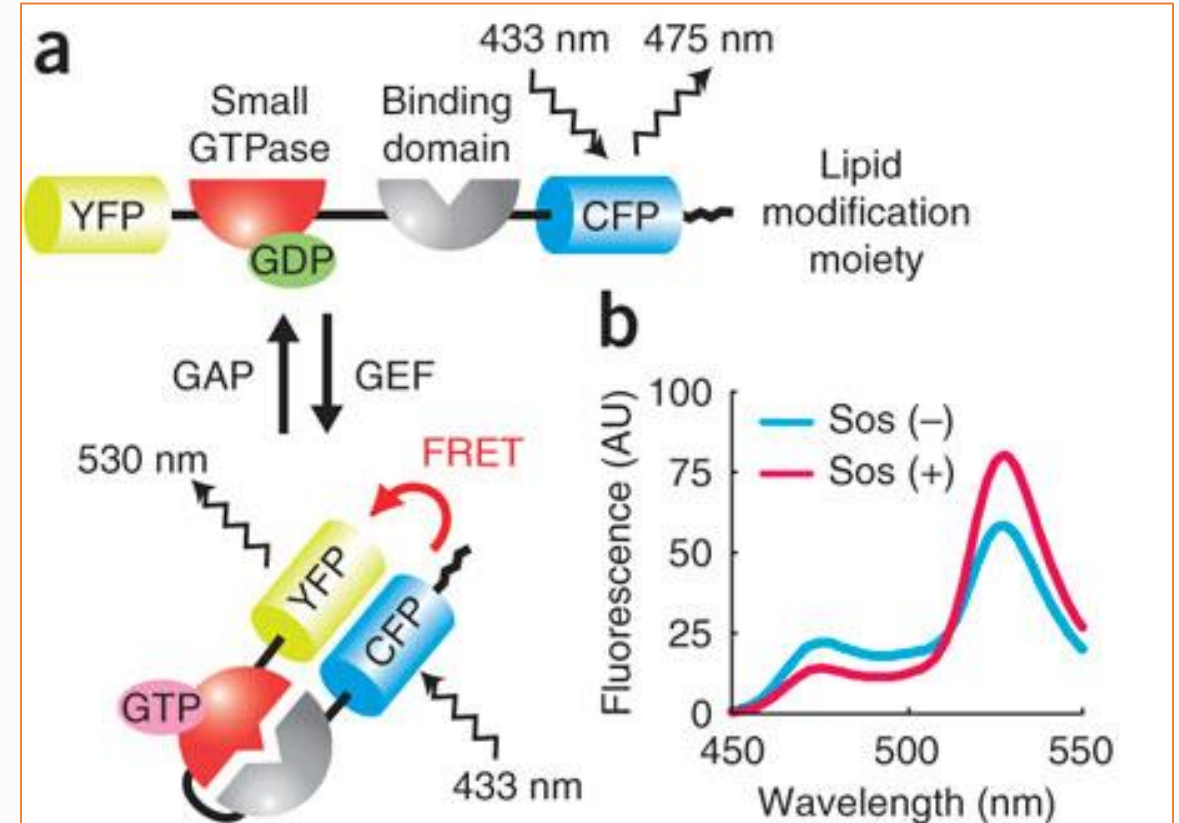
[19]

- Donor and acceptor fluorophores conjoined to the **same molecule**
- This type **preferred** because of :
 - High signal-to-noise ratio
 - Easy loading into the cells



❑ Intramolecular biosensor [19]

- Structure of an intramolecular FRET biosensor of small GTPase, Raichu



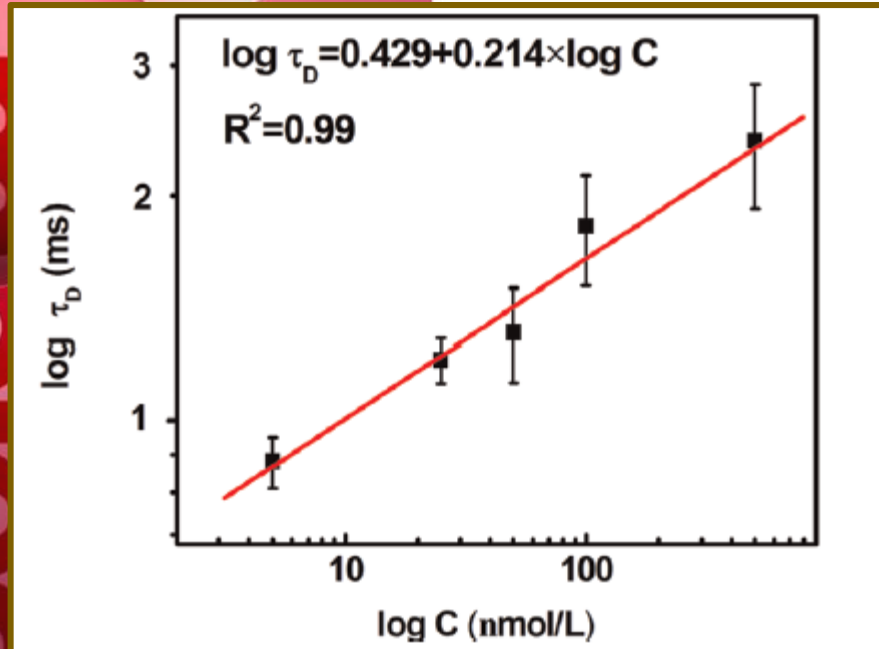
- 
- An aptamer-based biosensor for detection of thrombin using **fluorescent quantum dots** as labeling probes [20]

A B S T R A C T

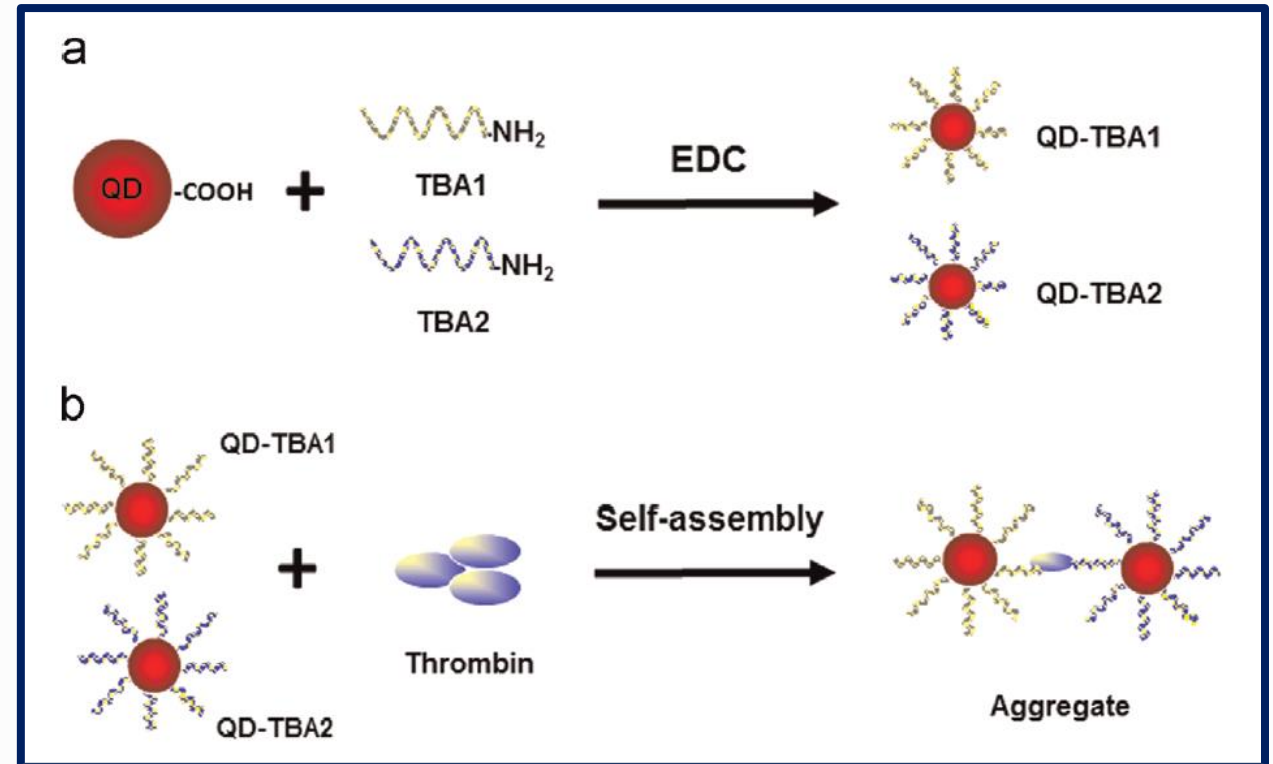
In this study, an aptamer-based single particle method was developed for the thrombin detection in human serum samples using **fluorescence correlation spectroscopy** (FCS). In this method, quantum dots (QDs) were used as the fluorescent probes and thrombin-binding aptamer (TBA) was used as molecular recognition unit. When two QDs probes labeled with **TBA (QD-TBA1 and QD-TBA2)** are mixed in a sample containing thrombin targets, the binding of targets will cause QDs to form dimers (or oligomers) with bigger sizes, which leads to the nearly double increase in the characteristic **diffusion time of QDs** in the detection volume of FCS. FCS method can detect the change in the characteristic diffusion time of QDs. Firstly, the diffusion and blinking behaviors of QD-TBA probes in the presence of thrombin were investigated by FCS and total internal reflection fluorescence microscopy (TIRFM) imaging system, and the experimental results documented that **QD-TBAs were bound together** with “one-by-one” structure **when thrombin were added** into the solution. And then, the assay conditions were optimized in order to improve the sensitivity and specificity of this method. Under the optimized conditions, the linear range of the method is from 5.0 nM to 500 nM of thrombin, and the limit of detection is about 2.6 nM. Finally, this method was applied to homogeneous determination of thrombin in human serum samples.

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□ An aptamer-based biosensor for detection of thrombin using **fluorescent quantum dots** as labeling probes [20]



The linear relationships between the logarithm of the characteristic diffusion time ($\log \tau_D$) of QDs and logarithm of thrombin concentration ($\log C$)



The procedure of aptamer-based single particle method for homogeneous detection of thrombin. QDs were linked with aptamer (TBA1 and TBA2) using EDC as coupling reagent (a). The QD-TBA probes reacted with the different concentrations of thrombin or human serum samples and formed the dimer (b).



❑ Surface Plasmon resonance (SPR)-based optical biosensors

[3]

✓ **SPR**: Collective oscillation of **conduction electrons** of in encounter with light

➤ **Plasmonic metal** nanostructures as optical-signal transducer

❖ Metals:

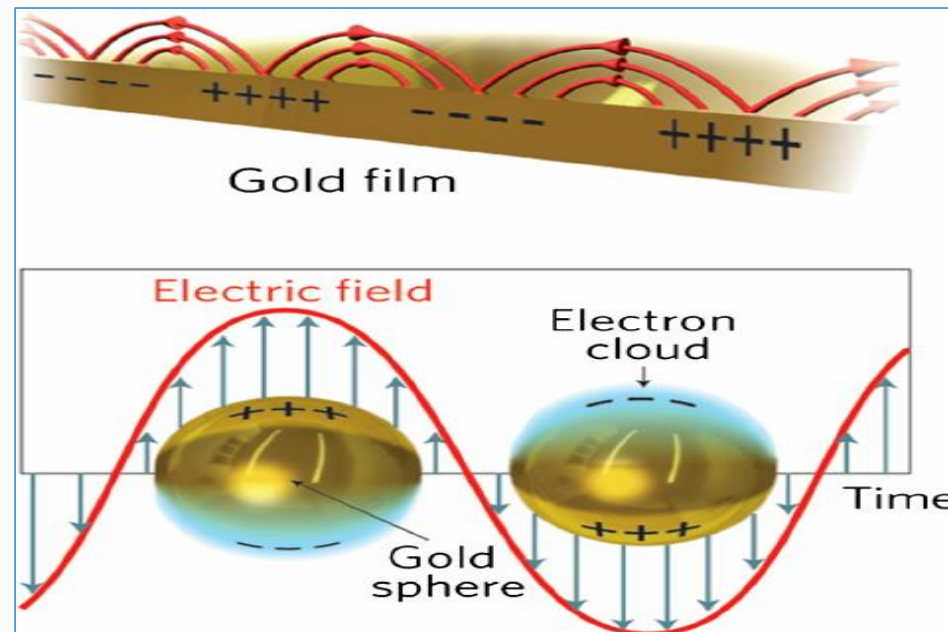
❖ Silver, copper, aluminum , gold and etc.

❖ **Gold** is preferred (chemical stability and free electron behavior)

□ Surface Plasmon resonance (SPR)-based optical biosensors

[3]

- Transduction surface can be:
- ✓ Thin metal film
 - ✓ Metal nanoparticle



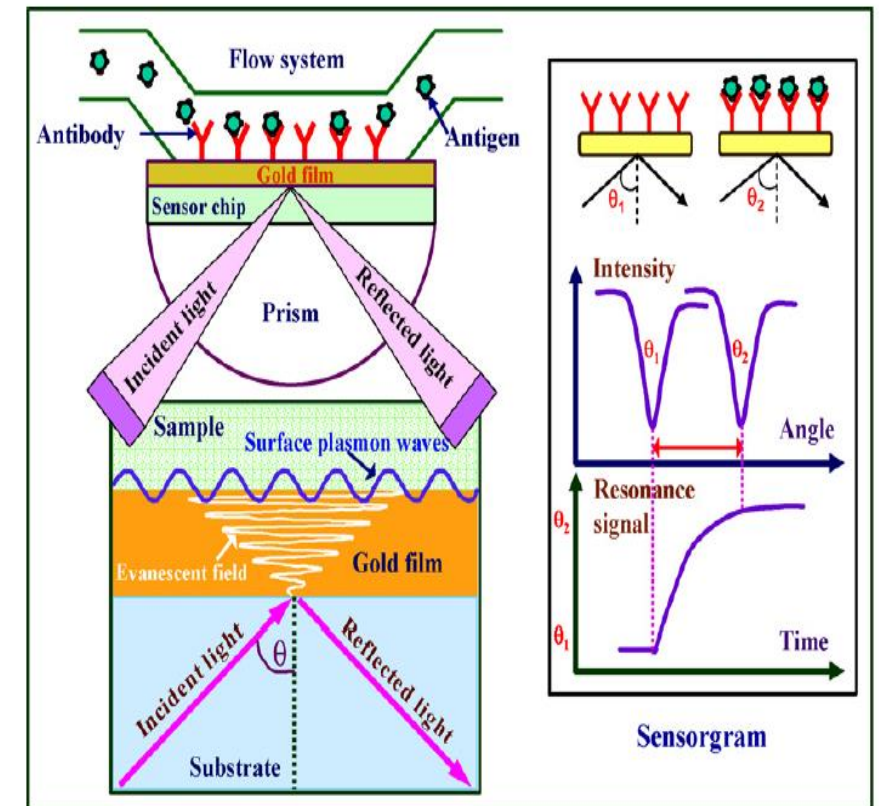


❑ Thin gold film SPR- biosensors [2]

- ❑ Is based on the **interaction of light** with a **thin metallic film/solution dielectric interface**
- ❑ Light of a certain wavelength impinges at a **specific angle** (SPR angle)
- ❑ Causing a **minimum in reflected light intensity**
(because of oscillations of mobile surface electrons)

❑ Thin gold film SPR- biosensors [2]

- Resonance angle is sensitive to refractive index change (caused by analyt binding)
- **Resonance angle shift** (arise from refractive index change)
- provide information on the:
 - ✓ **Amount** of bound analyte
 - ✓ Analyt –bioreceptor affinity



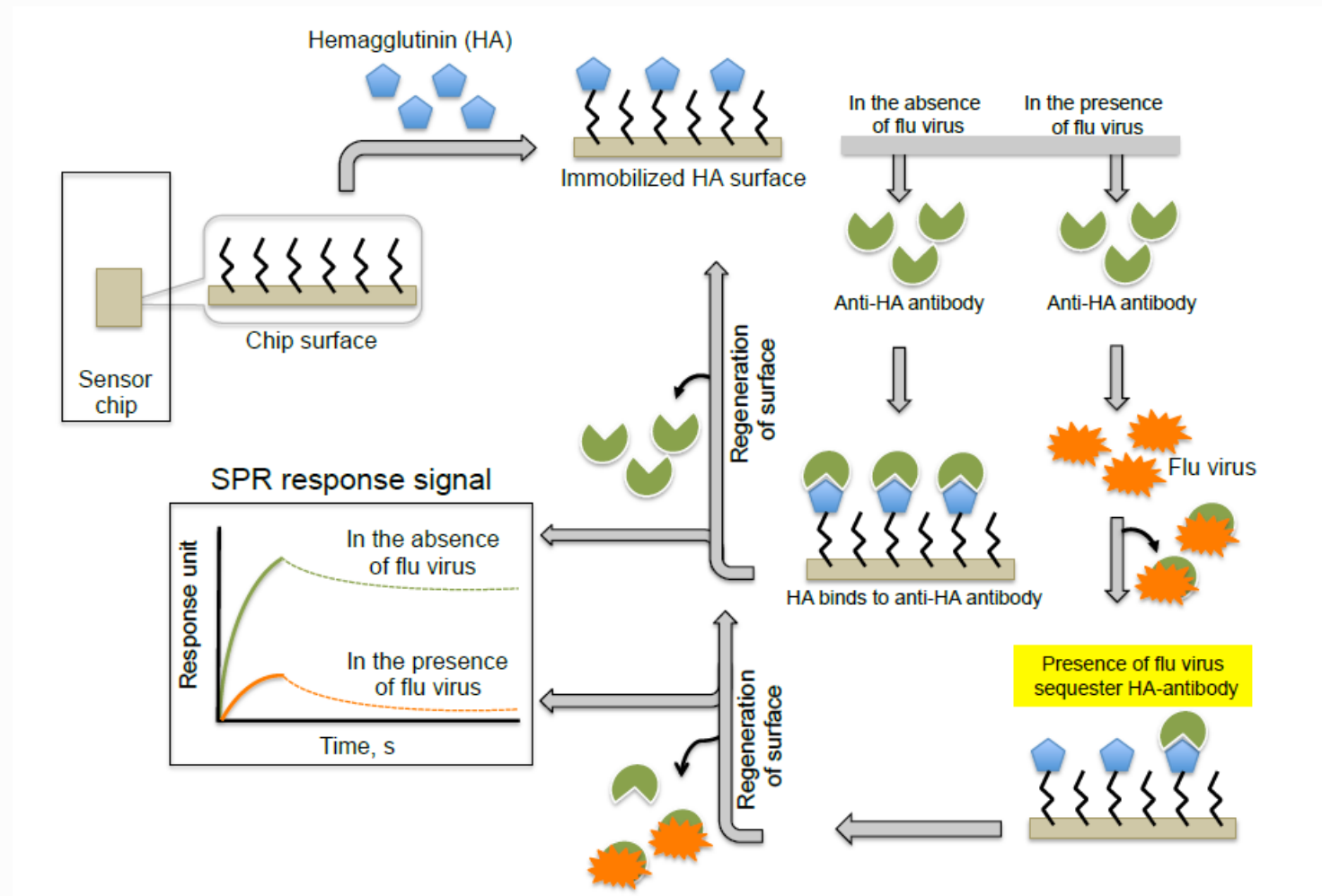


□ Thin gold film SPR- biosensors [21]

Abstract: Viral diagnosis and surveillance are necessary steps in containing the spread of viral diseases, and they help in the deployment of appropriate therapeutic interventions. In the past, the commonly employed viral detection methods were either cell-culture or molecule-level assays. Most of these assays are laborious and expensive, require special facilities, and provide a slow diagnosis. To circumvent these limitations, biosensor-based approaches are becoming attractive, especially after the successful commercialization of glucose and other biosensors. In the present article, I have reviewed the current progress using the biosensor approach for detecting intact viruses. At the time of writing this review, three types of bioreceptor surfaces (antibody-, glycan-, and aptamer-based) have been explored on different sensing platforms for detecting intact viruses. Among these bioreceptors, aptamer-based sensors have been increasingly explored for detecting intact viruses using surface plasmon resonance (SPR) and other platforms. Special emphasis is placed on the aptamer-based SPR platform in the present review.



□ Thin gold film SPR- biosensors [21]





❑ Metal nanoparticle SPR-based biosensors [22]

- ❑ Particles with dimensions **1-100 nm**
- ❑ In different forms of **spherical** , rod , triangular
- ❑ Are widely used in diagnostic fields because of their advantages such as:
 - High **surface/volume** ratio
 - Traceable **physicochemical properties** (related to their shape ,size and compound)
 - High stability



❑ Nanoparticle SPR-based biosensors [22]

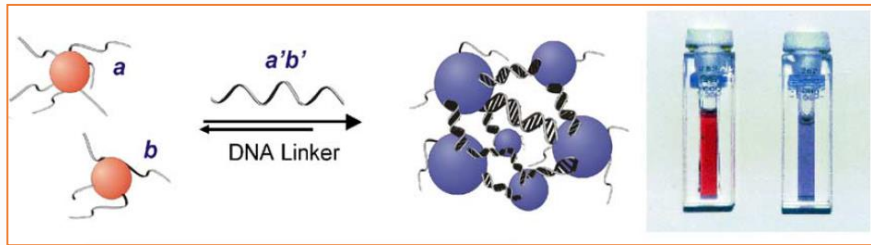
❖ **Gold nanoparticle** with:

- Strong absorption band in the **visible** region of the electromagnetic spectrum
- **Color change** associated with the size and SPR
- Provide **visual colorimetric** detection methods

□ Gold nanoparticle-DNA (AuNP-DNA) colorimetric [23]

Its based on the use of target DNA, complementary **Gold nanoparticle-modified probe**

❖ **Cross-linking (CL)** method: using **two type** of nanoprobe



❖ **Non-Cross-linking (NCL)** method: using **one nanoprobe** or interference of **bare nanoparticle** in presence probe

□ Direct detection of *Escherichia coli* genomic DNA using gold nanoprobe

[24]

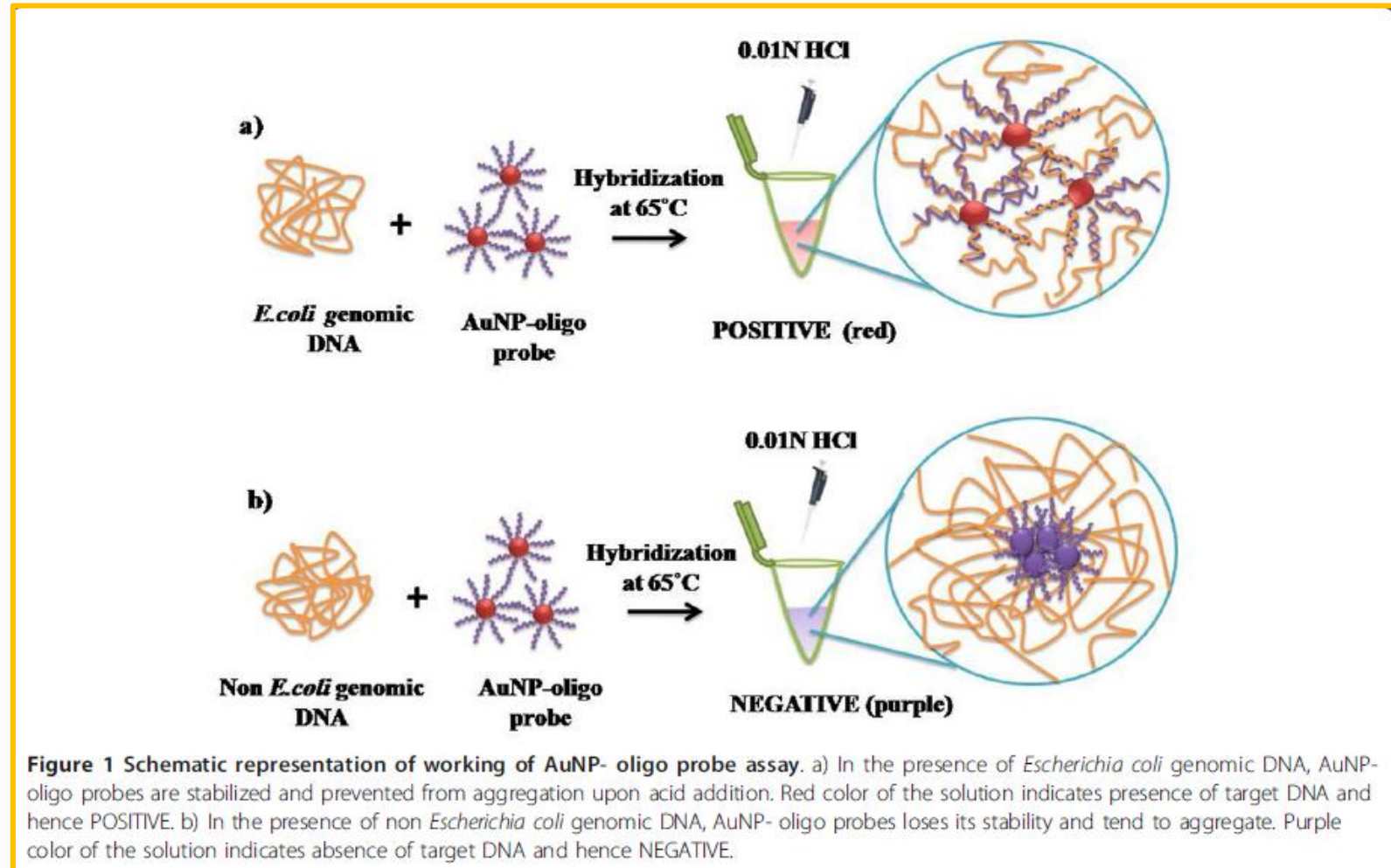


Figure 1 Schematic representation of working of AuNP- oligo probe assay. a) In the presence of *Escherichia coli* genomic DNA, AuNP-oligo probes are stabilized and prevented from aggregation upon acid addition. Red color of the solution indicates presence of target DNA and hence POSITIVE. b) In the presence of non *Escherichia coli* genomic DNA, AuNP- oligo probes loses its stability and tend to aggregate. Purple color of the solution indicates absence of target DNA and hence NEGATIVE.



❑ Conclusion

❑ Biosensors with:

- High **sensitivity** and **specificity**, of nanoscale size
- Cost-effective
- Using a small volume
- **Continuous** measurement
- High speed and
- Can be replaced with time-consuming and costly conventional methods to clinical diagnosis



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